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^{31}P NMR measurement of ATP synthesis rate in perfused intact rat hearts

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Using ^{31}P NMR and the saturation-transfer method, the unidirectional rate of ATP synthesis was measured in isolated, Langendorff-perfused, isovolumic rat hearts operating at a rate pressure product of 25.6 ± 2.5 (SE) $\times 10^3$ mmHg $\cdot$ min^{-1} and consuming O_2 at a rate of 35 ± 2 μmol $O_2 \cdot$ min$^{-1} \cdot$ (g dry wt)$^{-1}$, at 37°C. This rate was 7.2 ± 0.9 μmol $\cdot$ s$^{-1} \cdot$ (g dry wt)$^{-1}$ and was related to the rate of oxygen atom consumption by a ratio of 6.3 ± 0.9. These date show that in the intact heart the unidirectional rate of ATP synthesis exceeds the net rate of ATP synthesis and consumption by approximately a factor of 2.

^{31}P-NMR Saturation transfer ATPase (Rat myocardium)

1. INTRODUCTION

Magnetization-transfer techniques of NMR spectroscopy provide the unique capability of measuring unidirectional reaction rates in intact tissues during steady-state conditions (reviews [1–3]). Since the first application to measure the unidirectional rate of ATP synthesis by the *Escherichia coli* H^+-ATPase [4], the method of saturation transfer has been used to measure the rate of ATP synthesis in perfused hearts [5,6], kidneys [2] and suspension of yeast cells [7]. In intact hearts [5], the ATPase rate measurements by NMR were used to conclude that the P:O ratio in the myocardium is equal to the canonical value of 3, excluding a suggestion by Hinkle and Yu [8] that the P:O ratio in intact cells should be 2. However, the unidirectional rates measured by NMR need not be related to the O_2 consumption rate by the P:O ratio [3]. Furthermore, the measurements on the heart were not completely rigorous.

The calculation of the ATPase rate requires the determination of two parameters: first is the reduction in the intensity of the cytosolic P_i resonance when ATP_γ spins are saturated, and second is the T_1 of P_i determined either when ATP_γ is saturated or in the absence of the ATPase exchange while all other intracellular conditions that influence spin-lattice relaxation processes remain unaltered. Matthews et al. [5] measured the T_1 of the P_i resonance in hearts subjected to ~30 min of global ischemia and assumed it to be the T_1 in the absence of ATPase-catalyzed exchange between P_i and ATP. Although the ATPase reaction is indeed halted in the absence of oxygen delivery, ischemia drastically alters the cytosolic conditions including cytosolic P_i concentrations, pH, and ionic composition. Therefore it is difficult to assume that the T_1 obtained for P_i by this procedure can be used in the rate calculations as the spin-lattice relaxation time of this compound in the selective absence of the ATPase exchange in the myocardium.

The rates reported by Bittl and Ingwall [6] were obtained from a rigorous NMR determination; however, these investigators used an estimate for

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the P_i concentration which, as will be presented in this paper, is not valid for isovolumic, Langendorff-perfused rat hearts using glucose as the carbon substrate and operating at rate pressure products (RPP) in the range ~20 to ~50 × 10^3 mmHg/min.

Here we report ^{31}P NMR saturation-transfer measurements of the ATP synthesis rate in isovolumic, Langendorff-perfused rat hearts, where all parameters required for the rate calculations were obtained experimentally on each heart examined.

2. METHODS

The procedure for the preparation of the excised, isovolumic, Langendorff-perfused hearts was identical to that described [9]. Male Sprague-Dawley rats weighing ~300–400 g were used. Perfusion medium was a modified Krebs-Henseleit buffer [9] with 11 mM glucose as the only carbon source. For these experiments, the P_i was eliminated from the medium and replaced with equivalent amounts of KCl. The intraventricular balloon volume was adjusted to obtain a diastolic pressure of 4–8 mmHg. Hearts were paced at 300–330 beats per min if their spontaneous rhythm was slower than this rate. The intraventricular balloon contained a 100 mM solution of phenylphosphonate; the ^{31}P signal from this compound together with the balloon volume was used to calculate metabolite content. Myocardial oxygen consumption (MVO_2) was determined using an in-line, YSI oxygen electrode to monitor continuously the O_2 content of the effluent [9].

The general procedure for the saturation-transfer measurements was essentially identical to that described [9], except that only a single, low-power irradiation was used to saturate selectively ATP_γ. The progressive-saturation method was used to determine the T_1 of the P_i resonance while ATP_γ magnetization was nulled. It is important to note that, in order to minimize systematic errors due to frequency offset and imperfect 90° pulses, the spectrometer frequency was set within ~300 Hz of the P_i signal, and a composite 90° pulse was employed [10].

The points on the progressive-saturation measurement and the P_i intensity in the absence of ATP_γ saturation were obtained in a time-averaged fashion by cycling 24 times through the entire set of points, accumulating 12 scans per spectrum at each cycle. Two fully relaxed spectra without any selective irradiation were recorded before and after the kinetic measurements; these spectra were used to calculate the metabolite content of the hearts.

3. RESULTS

Fig.1 illustrates two spectra recorded with and without selective saturation of the ATP_γ from a typical kinetic measurement. The pulse-repetition time was 2.8 s in fig.1a and 5 s in fig.1b. The T_1 of P_i when ATP_γ is not saturated is ~1 s; it decreases typically to ~0.6 s upon saturation of ATP_γ. Therefore, the P_i resonance is fully relaxed in both fig.1a and b. However, other resonances such as the reference signal and ATP_γ are not. Therefore, a reduction in their intensity is observed under the rapid pulsing condition of fig.1b. The reduction in creatine phosphate (CP) resonance is

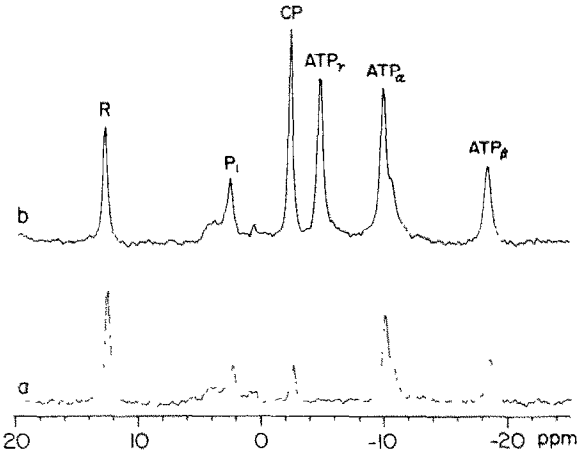

Fig.1. 146.1 MHz ^{31}P NMR spectra of a perfused rat heart recorded while ATP_γ resonance was selectively saturated (a), and while the selective irradiation frequency was moved downfield of the P_i resonance symmetrically opposite from the ATP_γ peak (b). Spectra were recorded using 90° pulses and 2.8 s repetition time for (a), and 90° pulses and 5 s repetition time for (b). Note that the P_i resonance is fully relaxed in both cases since saturation of ATP_γ reduces the P_i T_1 to less than 0.6 s. These spectra were taken from a series of spectra accumulated in a time-averaged fashion as described in section 2 for the measurement of the $P_i \longrightarrow ATP$ rate.

Peak R is the reference contained in the balloon.

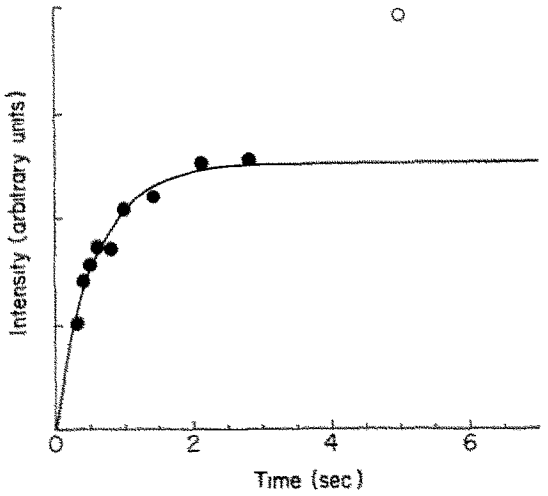

Fig.2. The intensities of the P_i resonance in a progressive-saturation sequence performed while ATP_γ is saturated (●) and in a fully relaxed spectrum recorded under the conditions of fig.1a (○). All data shown were obtained by cycling through each spectrum 24 times, accumulating 12 scans per spectrum during each cycle.

predominantly due to the creatine kinase-catalyzed exchange between CP and ATP_γ phosphates.

Fig.2 illustrates the data obtained for the rate calculation from a typical heart. P_i intensities (peak heights) were obtained from the progressive-saturation series performed while saturating ATP_γ and from a spectrum recorded when the selective irradiation frequency was moved downfield of the P_i resonance, symmetrically opposite from the ATP_γ peak. As previously mentioned, the P_i resonance was fully relaxed in the latter spectrum. The kinetic and the metabolic and mechanical data obtained from 6 hearts are given in tables 1 and 2, respectively.

4. DISCUSSION

Under the perfusion conditions employed in this study, the hearts operated at an MVO_2 of 36 ± 2 μmol $O_2 \cdot min^{-1} \cdot (g\ dry\ wt)^{-1}$ (table 2). This is comparable to the MVO_2 of the 'high' workload

Table 1

The kinetic data on the unidirectional ATP synthesis rate obtained from the saturation-transfer measurements

$\Delta M/M^0$	T_1* (s)	k_1 (s^{-1})	Flux (μmol·s^{-1}·(g dry wt)$^{-1}$)	Flux/MVO
0.34 ± 0.03	0.57 ± 0.05	0.64 ± 0.11	7.2 ± 0.9	6.3 ± 0.9

All values are means ± SE ($N = 6$). k_1, flux and flux/MVO were calculated for each measurement and then averaged to obtain the mean and the SE. $\Delta M/M^0$ is the fractional reduction in the P_i intensity upon saturation of ATP_γ. T_1* designates the T_1 of P_i measured while ATP_γ was saturated. Flux is the unidirectional rate for P_i incorporation into ATP; this is equal to $k_1[P_i]$. MVO is the rate of oxygen consumption in μgatom/s per g dry wt; MVO = (MVO_2/30) and flux/MVO ratio is dimensionless

Table 2

Mechanical and metabolic properties of the hearts used for the kinetic measurements

ATP content (μmol·(g dry wt)$^{-1}$)	P_i content (μmol·(g dry wt)$^{-1}$)	HR (min^{-1})	RPP[a] (10^3 mmHg/min)	MVO_2 (μmol O_2·min^{-1}·(g dry wt)$^{-1}$)
26 ± 3	11.9 ± 1.1	317 ± 8	25.6 ± 2.5	35 ± 2

[a] Rate pressure product; the product of heart rate and the peak systolic pressure

All values are means ± SE ($N = 6$). All data represent an average obtained over the duration of the period when saturation-transfer measurements were performed

group of Bittl and Ingwall [6] and somewhat higher than the 20 ± 1.2 μmol $O_2 \cdot min^{-1} \cdot$ (g dry wt)$^{-1}$ reported for the study by Matthews et al. [5]. It is seen that at this RPP and MVO_2, the unidirectional flux (which is simply the product of the P_i content and the pseudo-first order rate constant k_1 determined by NMR) is related to the rate of consumption of oxygen atoms with a ratio of 6.3 ± 0.9 (table 1). This is approximately twice as large as the ratio of 3.5 ± 0.8 reported by Matthews et al. [5]. The difference is predominantly due to the T_1 values used in these calculations; the fractional reductions observed by us and Matthews et al. [5] in the P_i intensity upon saturation of ATP_γ are essentially the same (0.34 ± 0.03 (table 1) vs 0.36 ± 0.02 [5]). Similarly, the P_i content of the hearts is also comparable (11.9 ± 1.1 (table 1) vs 9.7 ± 1.1 [5]). However, as previously mentioned, Matthews et al. [5] measured a T_1 of P_i in ischemic hearts and assumed this to be the T_1 of P_i in normoxic hearts in the selective absence of the ATPase exchange. Our T_1 determination was performed in normoxic hearts while ATP_γ was saturated; this is a rigorous procedure in saturation-transfer studies. From our data it is possible to calculate a T_1 for P_i in normoxic hearts in the selective absence of $P_i \leftrightarrows$ ATP exchange; this turns out to be approx. 1 s, as opposed to a T_1 of 2 s determined at 30 min post-ischemia [5]. It should be mentioned that subsequent to a 30 min ischemic period, we also obtained T_1 values ranging from 1.6 to 2.5 s for the myocardial P_i resonance.

Comparing our data with those of Bittl and Ingwall [6], it is seen that the T_1 of P_i while ATP_γ is saturated and the pseudo-first order rate constants are similar. However, the P_i content estimated by these investigators is approximately one-half of the P_i content directly measured by us under virtually identical workload conditions. It has been noted previously that the P_i/ATP ratio in a Langendorff-perfused heart using glucose as the substrate is ~0.4 [12]. The ATP content in the hearts used in our experiments was 26 ± 3 μmol $\cdot$ (g dry wt)$^{-1}$ (table 2). This is essentially the same as the previously reported values of 26–27 μmol $\cdot$ (g dry wt)$^{-1}$ [9,12]. Using the P_i/ATP ratio of 0.4 and ATP content of 26–27 μmol $\cdot$ (g dry wt)$^{-1}$, the calculated P_i content is 10–11 μmol $\cdot$ (g dry wt)$^{-1}$. This number, calculated on data from other studies, is essentially identical to the experimentally determined P_i level in this study (table 2).

In the saturation-transfer measurements of the $P_i \longrightarrow$ ATP rate, γ-phosphates of ATP (cytosolic plus mitochondrial) were saturated and the consequences on the P_i resonance were monitored. This P_i resonance arises predominantly from the cytosolic pool. Therefore, the observed effect is dependent on the activities of both the mitochondrial H^+-ATPase and P_i mitochondrial transport. The rate of incorporation of P_i into ATP by glycolysis is very slow compared to oxidative phosphorylation [11], and it is assumed that the cytosolic ATPases such as the myosin ATPase Na^+/K^+-ATPase operate predominantly in the ATP hydrolysis directions. If the exchange between cytosolic and mitochondrial P_i is rapid, these saturation-transfer measurements yield the unidirectional rate of ATP synthesis by the H^+-ATPase. Recent measurements of P_i transport rates in suspensions of mitochondria suggest this to be the case (Ogawa, S. and Lee, T.M., personal communications; 1985).

The $P_i \longrightarrow$ ATP rate determined by saturation transfer need not be related to oxygen consumption by the P:O ratio which is normally assumed to be 3. This follows from the fact that the saturation-transfer technique measures unidirectional rates. It is the net rate of ATP synthesis (i.e. the difference between the $P_i \longrightarrow$ ATP and ATP $\longrightarrow P_i$ unidirectional rates of the mitochondrial H^+-ATPase) that is related to oxygen consumption by the P:O ratio. Therefore, it is erroneous to assume that the saturation-transfer experiments can be used to determine the P:O ratio. The P:O ratio cannot be greater than the ratio of the unidirectional rate determined by NMR and the rate of oxygen atom consumption (designated as the flux/MVO ratio in table 1). However, only in the limit where ATP synthesis by the H^+-ATPase is occurring unidirectionally (i.e. there is no ATP $\longrightarrow P_i$ conversion by the H^+-ATPase) is the rate determined by saturation transfer indeed equal to the net rate of ATP synthesis and the flux/MVO ratio equal to the P:O ratio.

Our data demonstrate that in the intact heart operating at a moderate workload and O_2 consumption rate, the turnover of the H^+-ATPase is faster than the net ATP synthesis and consumption rate in the heart. Therefore, it is not possible to determine the P:O ratio in the intact myocardium

from NMR measurements conducted at this workload. If the P:O ratio in the myocardium is between 2 and 3, then the unidirectional rate of ATP synthesis is approx. 3–2-times as fast as the net rate of ATP synthesis and consumption. This conclusion has an interesting implication concerning the H^+-ATPase rates at different workloads. The RPP of $\sim 25.6 \times 10^3$ mmHg·min^{-1} (table 2) attained by the hearts used in our measurements corresponds to the lower end of the physiologic range. A rat heart can operate at higher RPP values and a correspondingly high MVO_2. Given the high unidirectional rate of the H^+-ATPase relative to the net ATP synthesis rate, this enzyme can meet up to 2- or 3- (corresponding to P:O ratio of 3 or 2, respectively) fold increase in the ATP demand of high workloads without having to increase its unidirectional flux. In other words, the H^+-ATPase activity need not be regulated by the workload and MVO_2 up to an MVO_2 of ~70 or 100 μmol·min^{-1}·(g dry wt)$^{-1}$ depending on whether the P:O ratio is 3 or 2, respectively. In this range, the increased ATP demand of the myocardium can be met as the unidirectional $P_i \longrightarrow$ ATP rate of the H^+-ATPase stays constant but the reverse rate in the ATP $\longrightarrow P_i$ direction diminishes to zero. This indeed appears to be the case, because preliminary data obtained at an MVO_2 of 74 ± 3.3 (SE, $N = 4$) μmol O_2·min^{-1}·(g dry wt)$^{-1}$ yielded essentially the same unidirectional $P_i \longrightarrow$ ATP flux (7.2 ± 1.7 [SE, $N = 4$]) as that obtained at an MVO_2 of 35 μmol O_2·min^{-1}·(g dry wt)$^{-1}$; at the higher level O_2 consumption the flux/MVO ratio was reduced to 2.9 ± 0.6 (SE, $N = 4$). It should be noted that ATP $\longrightarrow P_i$ activity of the mitochondrial H^+-ATPase does not constitute a futile ATP consumption. This reaction is coupled to electrogenic H^+ extrusion; the overall coupled reaction of proton translocation and ATP hydrolysis does not dissipate energy or the ATP pool since it is the proton electronmotive force that drives the ATP synthesis by this enzyme.

In summary, we conclude that the saturation-transfer measurements yield a unidirectional rate of ATP synthesis which is ~6-fold higher than the rate of consumption of oxygen atoms at an RPP of 25.6×10^3 mmHg·min^{-1}, indicating that the ATPase turnover of the H^+-ATPase must be 2–3-fold higher than the net rate of ATP consumption synthesis in the myocardium at this workload.

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